Ultrasonographic examination of the reticulum, rumen, omasum and abomasum during the first 100 days of life in calves

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Abstract: The goal of this study was to examine the development of the reticulum, rumen, omasum and abomasum in six calves from birth to 100days of age by means of six serial ultrasonographic examinations. The examinations were carried out in standing animals using a 5MHz-transducer as described previously. The calves were primarily fed milk until examination 4 and then they were weaned. The reticulum was assessed for its shape and contractility, the rumen for its size and content, the omasum for its size, content and motility and the abomasum for its size and content before and after the ingestion of milk. The reticulum was seen in all calves starting at examination 2 and had biphasic and triphasic contractions; the latter were associated with eructation. The rumen was always imaged in all calves as early as Day 1 and its visible size increased progressively in all intercostal spaces (ICSs) during the study period. The omasum was best imaged in the 8th or 9th ICS; it was seen medial to the liver dorsally and usually medial to small intestines ventrally. Its visible size in these two ICSs increased progressively but omasal motility was not apparent. In newborn calves the abomasum was the largest compartment and dominated the abdominal cavity. It was visible from the 5th ICS to the flank. Except for examination 2, the mean visible abomasal length was significantly larger after feeding than before. Lateral abomasal extension to the left was greater than to the right at examinations 1-4, but was much smaller than to the right at examinations 5 and 6 because of progressive expansion of the rumen. Abomasal extension into the right hemiabdomen changed little during the study period.

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ABSTRACT

The goal of this study was to examine the development of the reticulum, rumen, omasum and abomasum in six calves from birth to 100 days of age by means of six serial ultrasonographic examinations. The examinations were carried out in standing animals using a 5 MHz-transducer as described previously. The calves were primarily fed milk until examination 4 and then they were weaned. The reticulum was assessed for its shape and contractility, the rumen for its size and content, the omasum for its size, content and motility and the abomasum for its size and content before and after the ingestion of milk. The reticulum was seen in all calves starting at examination 2 and had biphasic and triphasic contractions; the latter were associated with eructation. The rumen was always imaged in all calves as early as Day 1 and its visible size increased progressively in all intercostal spaces (ICSs) during the study period. The omasum was best imaged in the 8th or 9th ICS; it was seen medial to the liver dorsally and usually medial to small intestines ventrally. Its visible size in these two ICSs increased progressively but omasal motility was not apparent. In newborn calves the abomasum was the largest compartment and dominated the abdominal cavity. It was visible from the 5th ICS to the flank. Except for examination 2, the mean visible abomasal length was significantly larger after feeding than before. Lateral abomasal extension to the left was greater than to the right at examinations 1 to 4, but was much smaller than to the right at examinations 5 and 6 because of progressive expansion of the rumen. Abomasal extension into the right hemiabdomen changed little during the study period.

Keywords: Cattle; Calf; Ultrasonography; Reticulum; Rumen; Omasum; Abomasum; First hundred days

1. Introduction

The ultrasonographic characteristics of the reticulum, rumen, omasum and abomasum have been described in detail in adult cattle and were summarised in a recent report (Braun, 2009). Similar studies have been carried out in milk-fed calves from birth to 20 days of age (Jung, 2002) and in 90-

day-old hay-fed calves (Gautschi, 2010). Comparison of the studies showed that the ultrasonographic findings varied greatly between these two age groups. The digestive tract of calves changes dramatically during the first few months when milk is replaced by hay, which leads to an increase in the size of the rumen. Biphasic contractions of the reticulum were seen in all calves in which the reticulum could be imaged ultrasonographically. Reticular motility was not as regular in milk-fed calves compared with hay-fed calves and adult cattle. In hay-fed calves, the amplitude of reticular contractions was significantly larger and the duration of the first contraction significantly shorter than in milk-fed calves (Gautschi, 2010). The size of the rumen was considerably smaller in milk-fed calves compared with hay-fed calves and the rumen occupied the entire left hemiabdomen in the latter. In an ultrasonographic study of 10 calves several days after birth, the omasum was seen in only one 14-day-old calf (Jung, 2002). In calves that were an average of 20 days of age, the omasum was seen in nine of ten, and by 90 days of age, the omasum could be seen in all 10 calves (Gautschi, 2010). Jung (2002) was able to see the omasal leaves in their entirety, whereas Gautschi (2010) reported seeing only the base of the omasal leaves. Ultrasonographic descriptions of the abomasum vary. In newborn calves, it was seen as a fluid-filled organ with echoic mucosal folds extending into the hypoechoic lumen (Jung, 2002). In milk-fed calves, the ultrasonographic appearance of the abomasum varied during ingestion of milk. The abomasum extended caudally as well as to the left and right side of the abdomen in a linear fashion with the volume of milk ingested (Wittek et al., 2005). Immediately after ingestion of milk, the contents of the abomasum appeared echoic. The milk rapidly formed a large echoic milk clot, which was broken down over the course of several hours resulting in liquefaction of the abomasal content (Miyazaki et al., 2009; Gautschi, 2010). The process of milk clotting was shown to differ between calves fed cow's milk (Gautschi, 2010) and calves fed milk replacer (Myiazaki et al., 2009). Studies by Jung (2002) and Gautschi (2010) involved groups of calves that were up to 14 days old, 16 to 33 days old and 87 to 90 days old. Studies using the same group of calves from birth to 100 days of age have not been carried out. The goal of this study was to investigate the ultrasonographic characteristics of the developing reticulum, rumen, omasum and

78 abomasum in six healthy calves from birth to 104 days of age. This is clinically relevant because
79 diseases such as ruminal drinking syndrome, abnormal milk clotting, abomasal ulcer and peritonitis
80 are common in calves at this age.
81
82 **2. Materials and methods**
83 *2.1. Animals*
84 Six clinically healthy newborn Holstein Friesian bull calves weighing 47.8 ± 8.01 kg (mean ±
85 sd) were enrolled in this study within one day of birth.
86 Six clinically healthy newborn Holstein Friesian bull calves were purchased from three dairy
87 farms immediately after birth and enrolled in the study. None of those farms had health problems.
88 The calves had received 2 litres of colostrum from the dam and weighed 47.8 ± 8.01 kg (mean ± sd).
89 Colostrum feeding was repeated twice at our clinic, and the calves were given 50 mg specific anti-*E.*
90 *coli* and polyvalent immunoglobulins (0.5 ml/kg body weight [bw], Locatim plus ad us. vet.®,
91 Biokema SA, Crissier), vitamin A, D_3 and E (5 ml, Aqua-Vit®, Werner Strickler AG, Zollikofen) and
92 selenium and vitamin E (1 ml/5 kg bw (Tocoselenit®, Dr. E. Graeub AG, Bern). In addition, the
93 calves received danofloxacin (1 ml/20 kg bw, Advocid® 2.5 %, Pfizer AG, Zürich) daily for five days
94 and 500 ml colostrum daily for 10 days. The colostrum had been obtained from each of the dams and
95 500-ml aliquots were stored at -20°C. The results of clinical, haematological and biochemical
96 examinations were within normal ranges and have been published elsewhere (Krüger, 2012). The
97 calves were bovine virus diarrhoea virus antigen negative.
98
99 *2.2. Study design*
100 The reticulum, rumen, omasum and abomasum were examined ultrasonographically as
101 described previously in detail (Gautschi, 2010; Braun et al., 2012; Krüger, 2012). The calves were
102 examined six times at three-week intervals (Table 1) and 3.0 to 5.5 hours after being fed cow's milk
103 at a rate of 12 % of body weight. The abomasum was also examined during and immediately after

104 feeding. The calves were weaned after examination 4 (62 days) and then fed hay high-quality second-
105 cut hay ad libitum.
106
107 *2.3. Ultrasound machine and video recorder*
108 A real-time B-mode ultrasound machine (EUB 8500, Hitachi Medical Systems, Zug) and a
109 linear or convex 5.0-MHz transducer (Type EUP L53) were used. The machine was connected to a
110 video recorder to record the motility of the reticulum, dorsal blind sac of the rumen and omasum, as
111 well as the process of abomasal filling during feeding.
112
113 *2.4. Preparation of calves and ultrasonographic examination*
114 The non-sedated calves were standing during the examinations. The calves were clipped on
115 both sides from behind the shoulder to the tuber coxae and from the transverse processes of the
116 thoracic and lumbar vertebrae to the ventral midline. The skin was cleaned with alcohol and lubricant
117 (Vetogel®, Streuli Pharma AG, Uznach) was applied. A contact gel (Aquasonic®, Polymed,
118 Opfikon/Glattbrugg) was also applied to the transducer.
119
120 *2.5. Technique of reticular ultrasonography*
121 The reticulum was examined in the ventral median or left paramedian area with the transducer
122 held parallel to the longitudinal axis of the animal. This allowed visualisation of the reticular apex
123 closest to the transducer and the abomasum or cranial dorsal blind sac of the rumen centrad. A 9-
124 minute video recording was made to assess reticular motility. The reticulum was also visualised at
125 each cranial intercostal space (ICS) from caudal to cranial and from dorsal to ventral on both sides.
126
127 *2.6. Technique of ruminal ultrasonography*
128 The rumen was examined at the 6th to 12th ICSs and the flank on both sides. Each ICS and the
129 flank were scanned from dorsal to ventral with the tranducer held parallel to the ribs. The size of the

rumen was determined by identifying the dorsal and ventral visible margins in each ICS analogous to the method described for goats (Braun et al., 2011). The dorsal and ventral visible margins of the rumen were determined by measuring the distance from each margin to the dorsal midline using a tape measure, and the size of the rumen was calculated by subtracting the distance of the dorsal margin from the distance of the ventral margin. The location of the longitudinal groove was identified, which served to determine the size of the rumen sacs. The dorsal rumen sac extends from the dorsal visible margin of the rumen to the longitudinal groove and the ventral sac extends from the longitudinal groove to the ventral visible margin of the rumen. The thickness of the ruminal wall was measured near the dorsal margin, the longitudinal groove and the ventral margin using a 13-MHz transducer. The dorsal blind sac of the rumen was scanned for 9 minutes with the transducer held parallel to the longitudinal axis of the calf and ventral to the left costal arch such that the transition from the reticulum to the blind sac was visible.

2.7. Technique of omasal ultrasonography

All ICSs on the right side were examined from dorsal to ventral with the transducer held parallel to the ribs; newborn calves were also scanned on the left side. The dorsal and ventral visible margins and the size of the omasum were determined analogous to the method used for the rumen. The visibility of the wall and leaves of the omasum were determined and a 9-minute video of the organ was recorded to assess its motility.

2.8. Technique of abomasal sonography

The abomasum was scanned at the level of the 5th and 12th ICSs and the flank on both sides starting at the ventral midline and progressing laterally and dorsally with the transducer held parallel to the ribs. The location and size of the organ and visibility of its wall, folds and contents were assessed. The distance between the xyphoid and cranial abomasal border and the abomasal length were measured and the visibility of the pylorus was determined. A video recording was made of the

156 abomasum slightly to the left of the ventral midline during ingestion of milk, and the time between
157 the start of milk intake and the appearance of the milk in the abomasum was measured using a stop
158 watch. The calves received 50 % of their daily milk ration, but no more than 4 litres, warmed to 39
159 °C, via a nipple connected to a rubber hose, which reached to the bottom of a 10-litre bucket.
160 Immediately after feeding, the position and size of the abomasum and its contents were re-assessed.
161
162 *2.9. Statistical analysis*
163 The statistical programme STATA 12 (StataCorp LP, Collage Station, Texas, USA, 2011) was
164 used to calculate means, standard deviations and frequency distributions. The Shapiro-Wilk test was
165 used to test the data for normal distribution. Differences in ruminal and abomasal size between
166 examinations and differences in ruminal wall thickness between different locations were analysed
167 using factorial analysis of variance (ANOVA) and a Bonferroni post-hoc test. The difference in
168 abdominal length before and after the ingestion of milk was analysed using a paired *t*-test. P d 0.05
169 was considered significant.
170
171 **3. Results**
172 *3.1. Ultrasonographic findings of the reticulum*
173 During examination 1, the reticulum was seen in only one calf at the 7th ICS on the right side.
174 From examination 2 onward, the reticulum was seen in the sternal region in all calves and up to
175 examination 4 was often displaced from the abdominal wall by the spleen or liver (Fig 1). After
176 examination 4, the reticulum was adjacent to the abdominal wall and the reticular wall was visible as
177 an echogenic line as described in adult cattle (Braun and Rauch, 2008). Because of its gaseous nature,
178 the reticular contents were not visible, or only produced an ill-defined echo near the reticular wall.
179 The only exception was one calf at examination 2, in which the reticular contents appeared
180 homogeneous and hypoechoic. Small projections indicating the mucusal folds were seen at all
181 examination times in one or two calves, and in one calf, the honeycomb pattern of the reticular wall

was apparent at the last examination. Reticular contractions had a biphasic pattern as seen in adult cattle, and typical triphasic contractions occurred in association with rumination (Braun and Rauch, 2008). The evaluation of reticular motility was possible in four calves and indicated a significant increase in the number of contractions during the study period ($P < 0.01$). The number of contractions per nine minutes was 7.8 ± 1.50 at examination 2, 8.8 ± 1.26 at examination 3, 10.8 ± 1.26 at examination 4, 12.7 ± 1.53 at examination 5 and 13.0 ± 1.0 at examination 6. The number of contractions per minute were 0.9 ± 0.17, 1.0 ± 0.14, 1.2 ± 0.14, 1.4 ± 0.14 and 1.4 ± 0.09 at the second to sixth examinations, respectively.

3.2. Ultrasonographic findings of the rumen

The rumen was visualised at each examination time in all the calves. In the caudal abdomen it was adjacent to the abdominal wall, and further cranially the spleen was seen between the abdominal wall and the rumen. At examination 1, the ruminal content was anechoic with hyperechoic stippling, and five calves had a small dorsal gas cap. Beginning at examination 2, most calves had reverberation artefacts dorsally, indicating a gas cap, and an ingesta phase ventrally. The transition between the two phases was characterised by the abrupt disappearance of the reverberation artefact. Because of their gaseous nature, the ingesta could not be visualised. The transition between the ingesta and the ventral fluid phase could not be seen in any of the calves.

The rumen was visible only from the left side at examination 1, but from both sides thereafter. The longitudinal groove appeared on the left as a mucosal fold as early as examination 1 (Fig. 2), separating the dorsal and ventral sacs of the rumen.

Because of superimposition of the lung and spleen, the distance between the dorsal visible margin of the rumen and the dorsal midline was largest in the 7th ICS (Fig. 3). It became smaller further caudally and was smallest in the 12th ICS. In the flank, the distance between the dorsal midline and dorsal margin of the rumen increased. In contrast, the distance between the dorsal midline and the ventral visible margin of the rumen did not change appreciably.

208 In each ICS, the overall size of the rumen as well as the size of the dorsal and ventral sacs
209 increased progressively with each examination (Table 2, Fig. 4). The most pronounced increase
210 occurred between examinations 4 and 5 after weaning. At examinations 1 to 3, the dorsal and ventral
211 sacs of the rumen were similar in size, but subsequently the ventral sac became slightly larger. The
212 relative and overall length of the rumen also increased gradually; at examination 1, the rumen did not
213 extend beyond the last rib, but was seen in the flank region thereafter.
214 The cranial dorsal blind sac of the rumen could not be seen at examination 1, but at subsequent
215 examinations appeared as a semicircular structure between the reticulum and ventral sac of the
216 rumen, similar to descriptions in adult cattle. It contracted immediately after the biphasic reticular
217 contractions.
218 The wall of the rumen was visible as an echoic line, and three layers including the serosal,
219 muscular and mucosal tunics could be clearly differentiated using the 13-MHz transducer. The mean
220 wall thickness ranged from 0.85 to 1.50 mm at the dorsal sac in the 12th ICS, which was not
221 significantly different from the other measuring sites at the longitudinal groove and ventral sac of the
222 rumen. Toward the end of the study period there was a slight but non-significant increase in wall
223 thickness.
224
225 *3.3. Ultrasonographic findings of the omasum*
226 The omasum was seen from the right side at each examination in all the calves, and from the
227 7th to 10th ICSs on the left side at examination 1 in three calves. It was medial to the liver dorsally
228 and usually medial to the small intestines ventrally, and only occasionally directly adjacent to the
229 abdominal wall. The omasum was seen on the right side at the 6th to 9th ICSs and occasionally at the
230 10th ICS during examinations 1 to 4; the best images were obtained at the 8th or 9th ICS. The omasal
231 wall appeared as a semi-circular to circular echoic line; it was completely circular in five newborn
232 calves. The omasal contents in these five calves were echoic and the omasal leaves appeared as fine
233 echoic bands. After examination 1, the omasal contents could not be seen because of their gaseous

234 nature, as described in adult cattle. On a few occasions, the origin of the omasal leaves remained
235 visible as echoic projections protruding into the omasal lumen.
236 The dorsal visible border of the omasum had a caudodorsal course (Fig. 5). It was furthest from
237 the dorsal midline at the 6th ICS and moved progressively closer to the dorsal midline toward the
238 10th ICS. The visible omasal size measured at the 8th and 9th ICSs increased from examinations 1 to
239 6, whereas the size measured in the 6th and 7th ICSs did not change appreciably (Fig. 6). With the
240 exception of examination 4, the visible omasal size was largest in the 8th and smallest in the 6th ICS.
241 Omasal motility was not detected in any of the calves.
242
243 *3.4. Ultrasonographic findings of the abomasum*
244 At examination 1, the abomasum was the largest organ and dominated the abdominal cavity. It
245 was visible at the 5th to 12th ICSs and from the ventral flank. The contents were heterogeneous and
246 hypoechoic with hyperechoic stippling and often contained hyperechoic particles of varying size
247 reflecting clotted milk. The abomasal wall appeared as a fine echoic line, and abomasal folds were
248 distinct in four calves. During examinations 5 and 6, the pylorus was visible on the right side parallel
249 to the fundic part of the stomach in four and six calves, respectively. The pylorus was seen in four
250 calves over all six examinations; it was oval to circular in cross-section and often had a characteristic
251 spoke wheel appearance (Jung, 2002).
252 The influx of milk was readily seen during feeding and first appeared as a cloud-like
253 hyperechoic mass. This expanded to fill the entire abomasal lumen and was seen as a homogeneous
254 hyperechoic content with a snowstorm appearance. The mean interval between the start of nursing
255 and the ultrasonographic appearance of milk in the abomasum varied from 10.2 to 24.4 seconds
256 (range of individual examinations, 5.9 to 68.0 sec). Within a few minutes, the homogeneous echoic
257 content underwent changes as previously described in milk-fed calves (Gautschi 2010). There was
258 formation of a hypoechoic peripheral zone with moving hyperechoic stippling and a large
259 homogeneous echoic clump centrally. After feeding, the echoic abomasal folds were always distinct

and easily differentiated from the surrounding content. A dorsal gas cap was seen after each feeding in one calf and after one or two feedings in four others as evidenced by reverberation artifacts on ultrasonograms. The pylorus was much more difficult to identify after feeding than before feeding, but was always located in the right hemiabdomen.

The abomasum was visible in the ventral midline from 0 to a maximum of 12.5 cm caudal to the xyphoid, and its mean length before feeding varied from 12.9 and 23.8 cm (Fig. 7). The visible abomasal length changed little from examinations 1 to 4, but at examination 6, was significantly greater than at examination 1 ($P < 0.05$). At examinations 1, 3 and 4, the visible abomasal length was greater before feeding than after feeding ($P < 0.05$).

At examination 1, the abomasum was visible on both sides of the ventral midline in five calves and only on the left in the remaining calf. It was visible on the left from the 5th ICS to the caudal flank and on the right from the 5th ICS to the cranial flank (Fig. 8). Extension to the left varied from 8.9 to 15.1 cm and to the right from 3.1 to 17.1 cm before feeding. After feeding, extension to the left and right varied from 8.9 to 23.2 cm and from 3.4 to 19.8 cm, respectively. Similar measurements were made at examinations 2 to 6; the details have been published elsewhere (Krüger, 2012).

Lateral abomasal extension was greater to the left than to the right at examinations 1 to 4, but much smaller to the left at examinations 5 and 6 because of the expanding rumen. The visible extension to the right changed little during the study period. The measurement made at the 10th ICS is shown as an example (Fig. 9).

4. Discussion

The reticulum was identified in only one of the newborn calves, presumably because it was very small and not adjacent to the abdominal wall. Thereafter the reticulum was visible in all calves and had typical biphasic contractions at all examinations. The reticulum was visible ultrasonographically in seven of ten three-week-old, milk-fed calves and also had biphasic contractions in a previous study (Gautschi, 2010). These findings were in slight contrast to

286 information that cyclic reticular contractions are first seen in calves at the age of six to eight weeks
287 (Dirksen, 2006).

288 The rumen was always seen at all examinations, in contrast to observations by Jung (2002) that
289 in calves up to 14 days old only the abomasum and omasum are visible ultrasonographically.
290 Although small and without apparent function, the rumen was seen containing variable amounts of
291 fluid as early as the first examination. The fluid is thought to consist mainly of respiratory secretions
292 and saliva (Berg, 1982) and possibly also milk; approximately 10 % of ingested milk flows into the
293 rumen because of incomplete oesophageal groove closure (Ruckebusch and Kay, 1971). From
294 examination 2 onward, the ultrasonographic appearance of the rumen was analogous to that described
295 in adult cattle. Warner and Flatt (1964) described the development of the forestomachs and
296 abomasum in calves on the basis of their tissue weight. The onset of roughage intake was associated
297 with a rapid increase in the weight of the reticulorumen and a decrease in the weight of the
298 abomasum. Similar changes were reported to start in calves at the age of three to four weeks (Dyce et
299 al., 2002). Tamate et al. (1962) found that there was a major development in the reticulorumen in the
300 first four weeks of life in calves fed milk, hay and grain. In the present study, there was progressive
301 expansion of the rumen caudally from examinations 1 to 4, and at examinations 5 and 6 the rumen
302 could be seen to reach into the right hemiabdomen. In this study, the largest increase in size of the
303 rumen occurred between examinations 4 and 5.

304 Similar to the rumen, the omasum was seen in all the calves at all examinations. This finding
305 was in contrast to the results of an earlier study in which the omasum was seen in only one of ten
306 calves during the first two weeks of life (Jung, 2002). The omasum appeared as a small spherical
307 structure in the right hemiabdomen medial to the liver. In four calves it could also be seen from the
308 left side. Good ultrasonographic visibility was attributable to a relatively narrow abdomen, small
309 rumen and liquid ruminal contents. The omasal contents were hyperechoic, and the omasal leaves
310 appeared as echoic bands in newborn calves. However, at subsequent examinations, the omasal
311 contents could not be visualised, similar to findings in adult cattle (Braun and Blessing, 2006). The

omasal leaves have been described as distinct echoic structures within the anechoic omasal contents in eight calves that were 12 hours to two weeks of age (Jung, 2002). However, in the present study, in agreement with Gautschi (2010), the omasal leaves were only recognisable as small projections on the luminal aspect of the omasal wall, or not at all after examination 1. Omasal size increased considerably between examinations 1 and 6, which was expected as part of the normal growth and development of the forestomachs. In contrast, Tamate et al. (1962) did not observe a substantial increase in omasal size during the first 12 weeks of life.

The abomasum was visualised in all the calves at all examinations, which was in agreement with other reports (Jung, 2002; Gautschi, 2010). As described in a previous study (Gautschi 2010), more of the abomasum was in the left hemiabdomen than in the right 3.5 to 5.5 hours after ingestion of milk. The visible laterolateral extension of the abomasum decreased from examinations 1 to 6; in the 5th to 9th ICSs on the left, the greatest decrease occurred between examinations 4 and 5, and caudal to the 9th ICS, the greatest decrease was between examinations 1 and 2. The greatest decrease in the lateral abomasal extension to the right occurred between examinations 1 and 2. Most of these decreases can be explained by progressive superimposition of the abomasum by the rumen and intestines, but the slight decrease in the visible left lateral abomasal extension documented at examinations 5 and 6 and the concurrent increase in right lateral extension in some ICSs indicate a slight change in position to the right. In the study by Tamate et al. (1962), most of the abomasum was displaced to the right hemiabdomen by the rapidly growing rumen as early as the 12th week of life. In adult cattle, the abomasum is located predominantly in the right hemiabdomen because of the rumen on the left. Maximum expansion of the abomasum, when it extends from the diaphragm to the pelvic inlet and from the ventral abdominal floor half way up the flanks, occurs after ingestion of a large volume of milk (Berg, 1982; Dyce et al., 2002). The pylorus was more readily identified in younger calves than in older ones. In adult cattle, the pylorus is difficult to visualise (Braun et al., 1997) because of superimposition of small intestines (Wittek et al., 2005). Conclusive identification of the pylorus is only possible when it is immediately adjacent to the abdominal wall and seen in cross-

section. The pylorus was only rarely seen after feeding in the present study as well as in an earlier investigation because it moves dorsally and to the right during filling of the abomasum with milk (Lischer, 1991).

5. Conclusion

The results of the present study documented changes in the forestomachs and abomasum in calves during the first 100 days of life by means of ultrasonography. Most notably, the reticulum was consistently imaged from examination 2 onward (day 16 to 23) and had a normal contractility pattern. The rumen expanded considerably after weaning and the visible size of the abomasum decreased progressively because of superimposition by the rumen and small intestines. These findings provide reference values for the examination of the forestomachs and abomasum for veterinarians in research and clinical practice concerned with digestive disorders in calves (ruminal drinking syndrome, abomasal ulcer, omasal reflux because of proximal ileus or abomasal displacement and abnormal milk clotting).

6. Conflict of interest statement

The authors of this paper have no financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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399 **Legends to figures**

400 Figure 1: Ultrasonogram of the reticulum in a 44-day-old Holstein Friesian calf obtained from the left
401 paramedial sternal region using a 5 MHz convex transducer. 1 Ventral abdominal wall, 2 Reticulum,
402 3 Abomasum, 4 Spleen, Cr Cranial, Cd Caudal.

403

404 Figure 2: Ultrasonogram of the rumen in a 44-day-old Holstein Friesian calf obtained from the 11th
405 intercostal space of the left side using a 5 MHz convex transducer. 1 Lateral abdominal wall, 2 Wall
406 of the dorsal sac of rumen, 3 Longitudinal groove, 4 Wall of the ventral sac of rumen, Ds Dorsal, Vt
407 Ventral.

408

409 Figure 3: Dorsal and ventral visible margins of the rumen imaged from the 7th intercostal space to the
410 caudal flank on the left at examination 4 (mean ± standard deviation) in six Holstein calves.

411

412 Figure 4: Size of the dorsal and ventral ruminal sacs and the entire rumen imaged from the left at the
413 11th intercostal space during the study period in six Holstein calves (mean ± standard deviation).

414

415 Figure 5: Dorsal and ventral visible margins of the omasum imaged from the right at the 6^{th} to 10^{th}
416 intercostal spaces at examination 3 (mean ± standard deviation) in six Holstein calves

417

418 Figure 6: Visible size of the omasum imaged from the right at the 6th to 9th intercostal spaces during
419 the study period (mean ± standard deviation) in six Holstein calves.

420

421 Figure 7: Length of the abomasum imaged from the ventral midline before and after ingestion of milk
422 during the study period (mean ± standard deviation) in six Holstein calves. There are no post-feeding
423 measurements at examinations 5 and 6 because of weaning of the calves.
424 * Difference between before and after feeding ($P < 0.05$; paired t-test)

[a] Difference between examinations 1 and 6 ($P < 0.05$; paired t-test).

Figure 8: Laterolateral extension of the abomasum before and after ingestion of milk imaged from the ventral abdominal wall from the 5th intercostal space to the flank (means) in six newborn Holstein calves.

Figure 9: Laterolateral extension of the abomasum before and after ingestion of milk imaged from the ventral abdominal wall at the level of the 10th intercostal space during the study period (mean ± standard deviation) in six Holstein calves. *, ** Difference between left and right ($P < 0.05$ and < 0.01, respectively), † Differences between examinations 4/5 and 4/6 ($P < 0.05$).

Table 1

Age of calves, interval between ingestion of milk and examination and amount of milk fed (mean ± sd, range in brackets) at six sonographic examinations during the first 100 days of life.

Examination	Age (days)g	Hours after feeding	Amount of milk fed (litres)
1	1.9 ± 1.14 (1.0 • 4.0)	4.6 ± 0.57 (3.0 • 5.5)	1.7 ± 0.50 (1.0 • 3.0)
2	19.7 ± 1.79 (16.0 • 23.0)	4.7 ± 0.43 (4.0 • 5.5)	2.2 ± 0.27 (2.0 • 3.0)
3	41.0 ± 0.76 (40.0 • 43.0)	4.9 ± 0.38 (3.5 • 5.5)	2.6 ± 0.42 (2.0 • 3.0)
4	62.1 ± 1.08 (60.0 • 64.0)	4.7 ± 0.60 (3.0 • 5.5)	2.6 ± 0.44 (2.0 • 4.0)
5	82.8 ± 1.03 (81.0 • 85.0)	weaned	weaned
6	99.2 ± 3.08 (95.0 • 104)	weaned	weaned

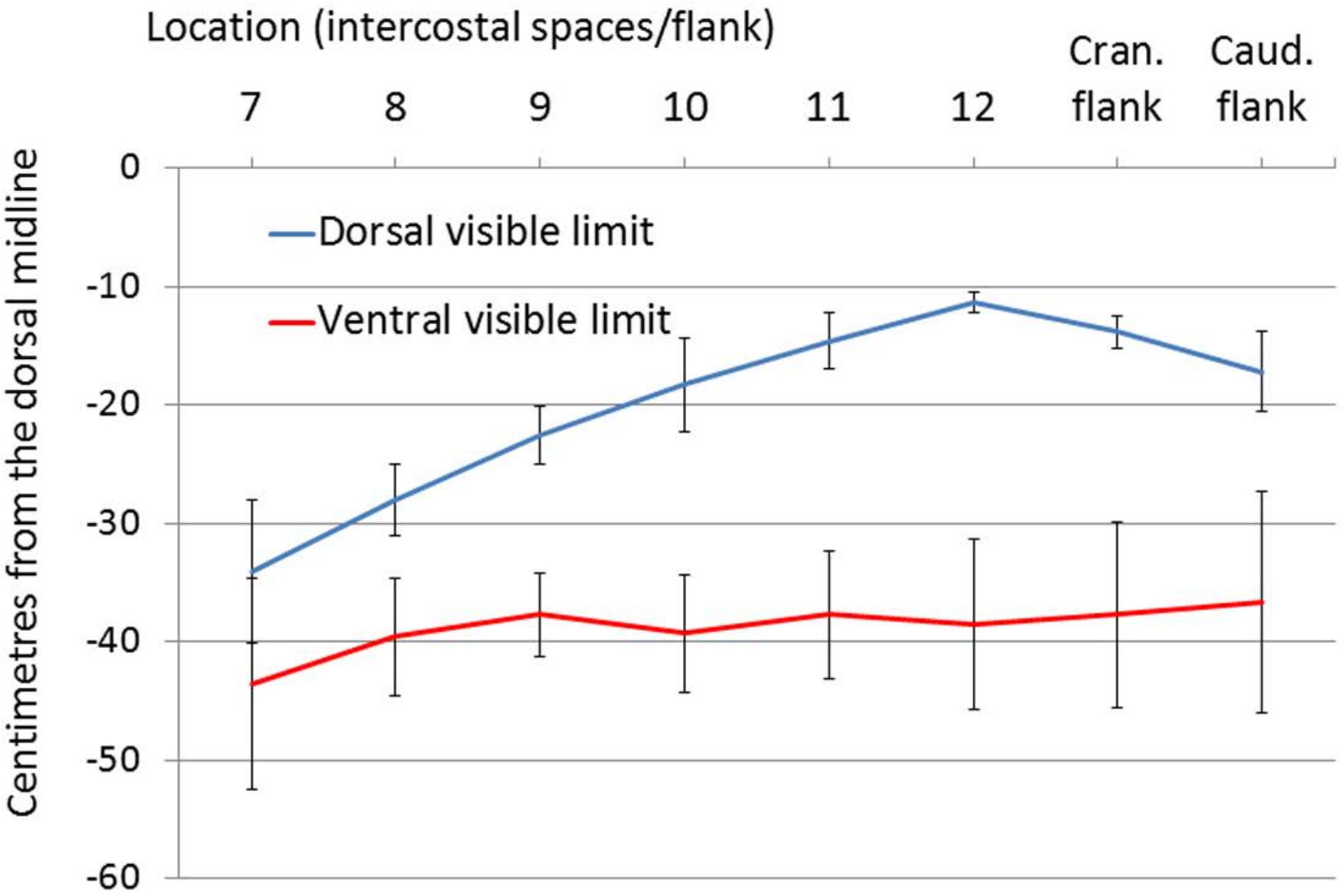
Table 2

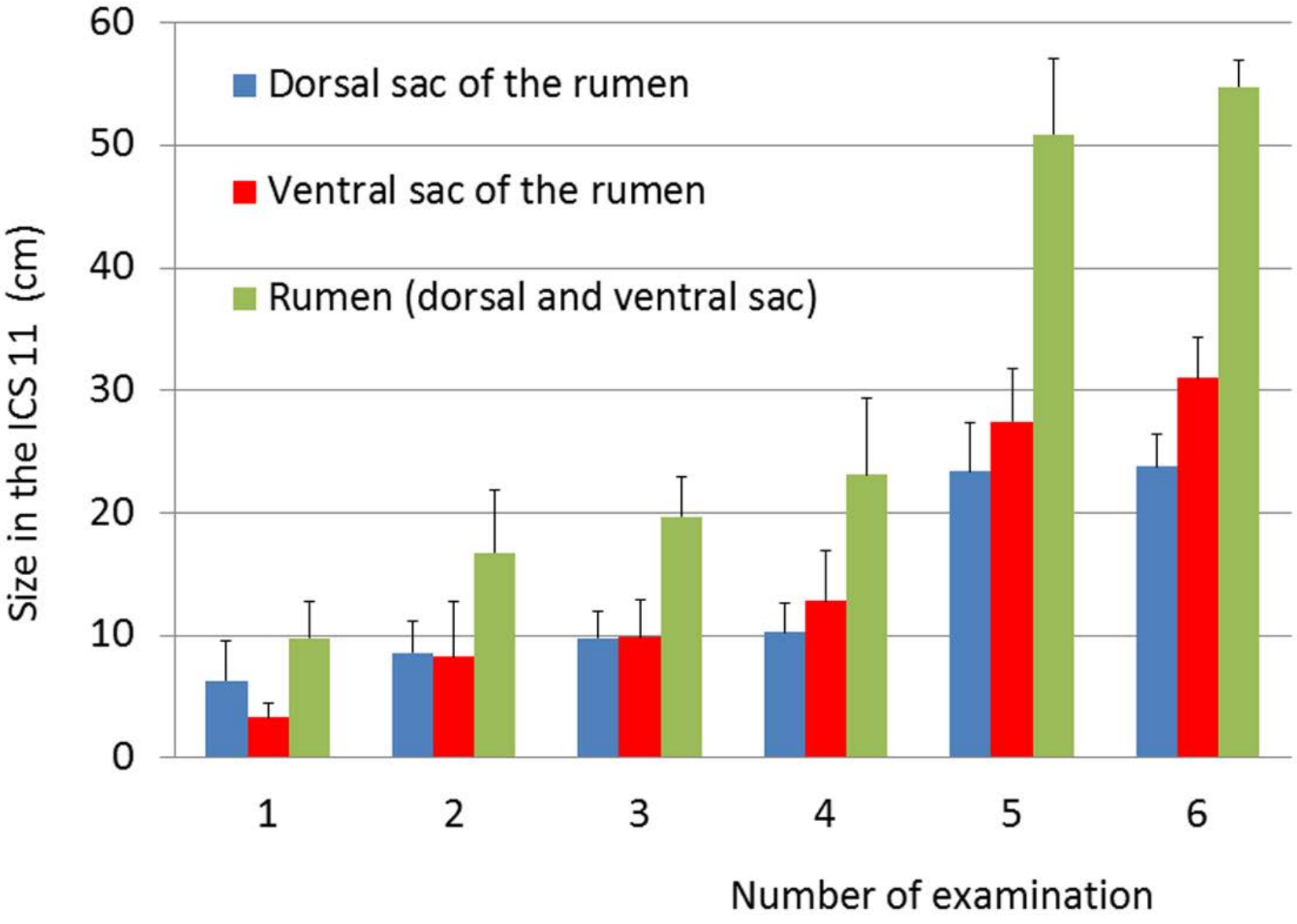
Left lateral extension of the rumen (cm) at the 6th to 12th intercostal spaces and the flank (mean ± sd, range in brackets) in six healthy Holstein calves.

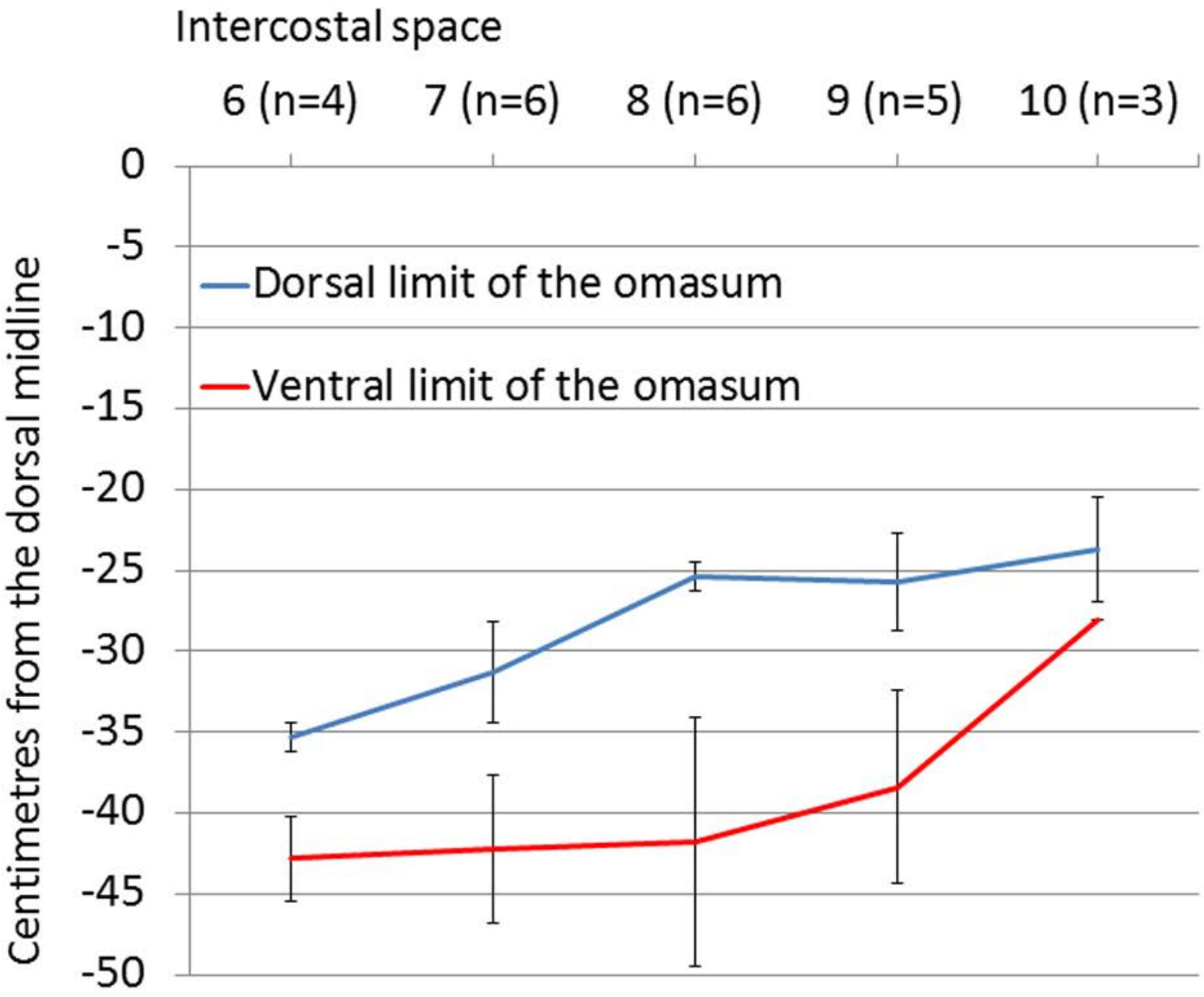
Site	Examination					
	1	2	3	4	5	6
6th ICS	-	-	-	-	9.5 ± 0.71 (9.0 – 10.0)	-
7th ICS	4.0 ± 2.83 (2.0 • 4.0)	5.3 [1] ± 2.87 (2.0 • 9.0)	5.9 ± 2.72 (2.5 – 10.0)	9.5 ± 3.92 * (5.0 – 13.5)	15.8 ± 3.63 * (10.5 – 19.5)	15.8 ± 1.99 (12.5 – 18.5)
8th ICS	4.6 ± 3.49 (0.50 • 10.0)	10.6 ± 3.46 (4.5 • 14.0)	11.1 ± 2.22 (8.5 – 14.0)	11.6 ± 2.48 * (8.0 – 14.0)	25.1 ± 4.12 * (17.5 – 30.0)	27.8 ± 5.22 (21.5 – 36.0)
9th ICS	5.7 ± 1.97 (3.5 • 8.5)	13.1 ± 4.07 (10.0 • 20.5)	12.0 ± 3.24 (9.0 – 16.5)	15.1 ± 2.08 * (12.5 – 17.5)	38.0 ± 5.34 * (28.0 – 43.5)	41.0 ± 5.66 (35.0 – 49.0)
10th ICS	9.8 ± 2.77 (5.0 • 12.5)	14.5 ± 4.31 (10.0 • 21.5)	15.8 ± 4.46 (11.0 – 22.0)	21.0 ± 6.35 * (13.0 – 29.0)	44.8 ± 7.57 * (32.5 – 52.3)	48.5 ± 3.85 (41.5 – 52.5)
11th ICS	9.8 ± 2.96 (6.0 • 14.0)	16.8 ± 5.07 (9.5 • 23.5)	19.7 ± 3.24 (16.0 – 24.0)	23.1 ± 6.31 * (16.0 – 31.0)	50.9 ± 6.25 * (38.5 – 56.0)	54.8 ± 2.18 (52.0 – 57.5)
12th ICS	8.8 ± 3.35 (4.5 • 12.5)	17.5 ± 5.46 (10.0 • 25.5)	20.2 ± 4.23 (14.5 – 26.0)	27.1 ± 7.10 * (20.5 – 36.5)	54.8 ± 4.83 * (46.0 – 59.0)	58.5 ± 2.44 (55.5 – 61.5)
Cranial flank	3.8 ± 1.76 (2.0 • 5.5)	14.1 ± 6.10 (9.5 • 24.5)	18.4 ± 4.75 (13.5 – 27.0)	23.8 ± 7.67 * (16.5 – 36.0)	50.4 ± 4.41 * (45.0 – 57.5)	54.0 ± 3.41 (49.0 – 57.8)
Caudal flank	-	14.0 ± 4.95 (10.5 • 17.5)	17.8 ± 7.46 (9.0 – 27.0)	23.0 ± 9.30 * (13.0 – 33.0)	45.3 ± 2.81 * (41.5 – 47.5)	48.1 ± 5.07 (40.5 – 55.0)

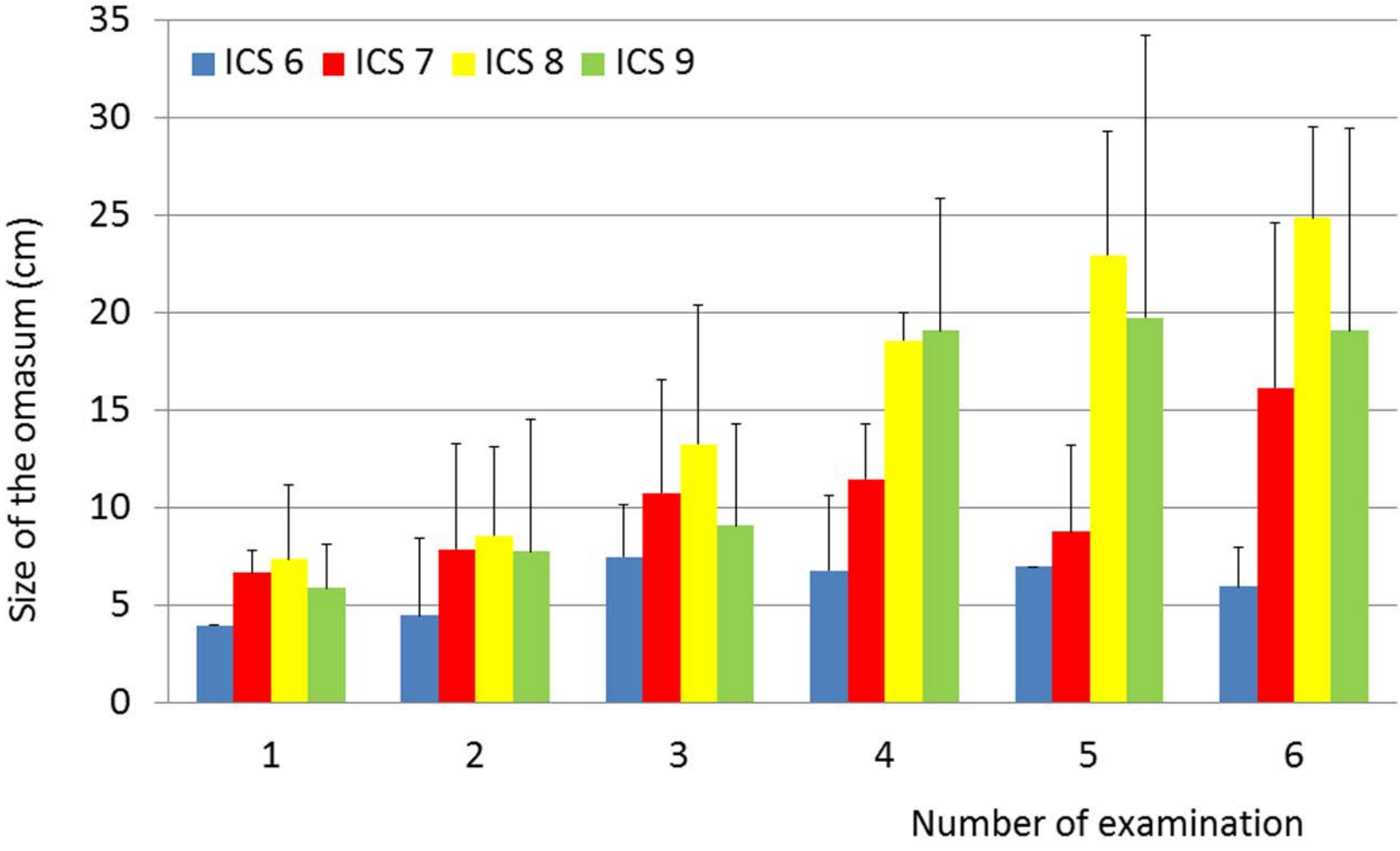
* Comparison with examination 4; P d 0.05 (ANOVA, Bonferroni test)

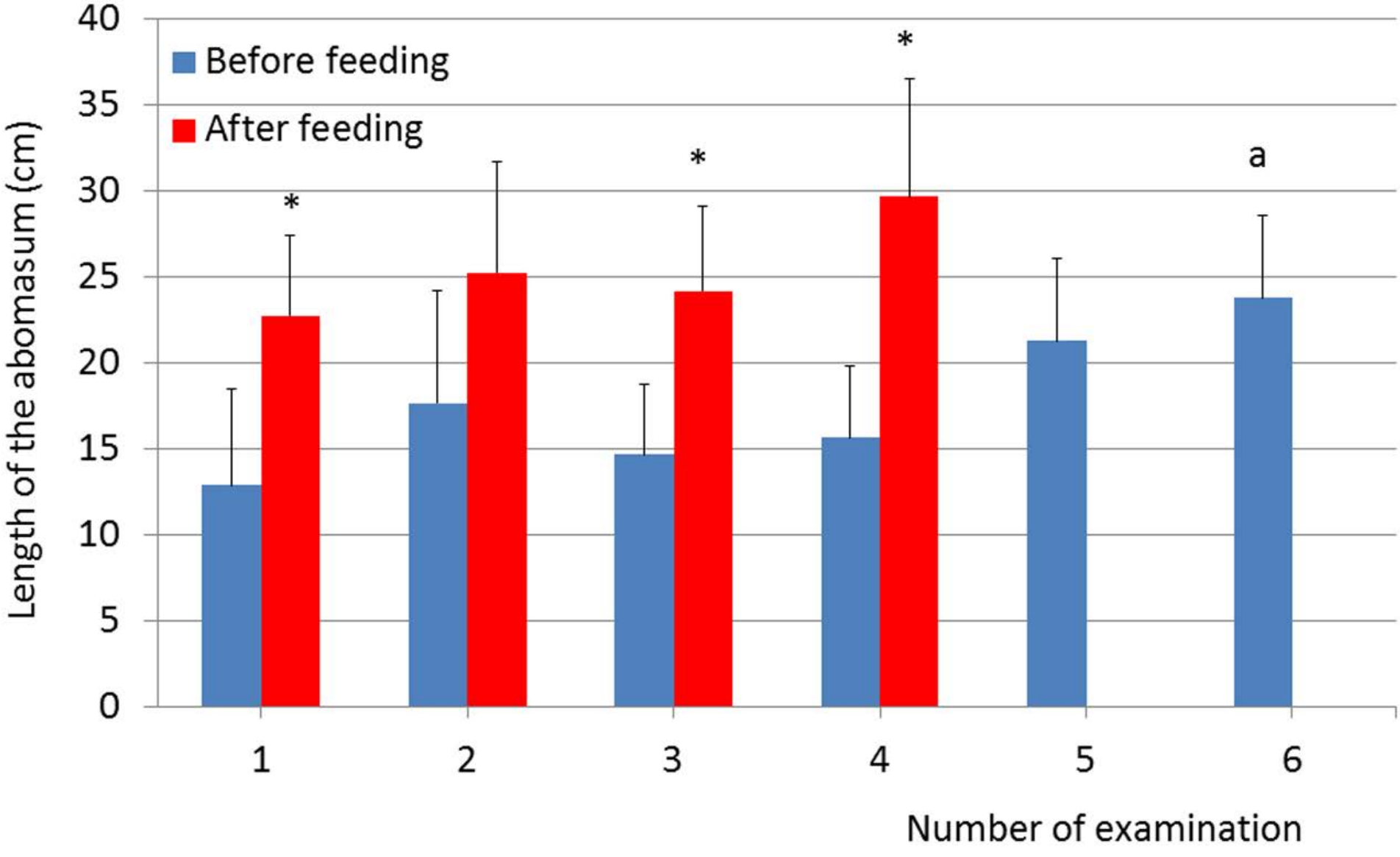
[1] Median (no normal distribution)

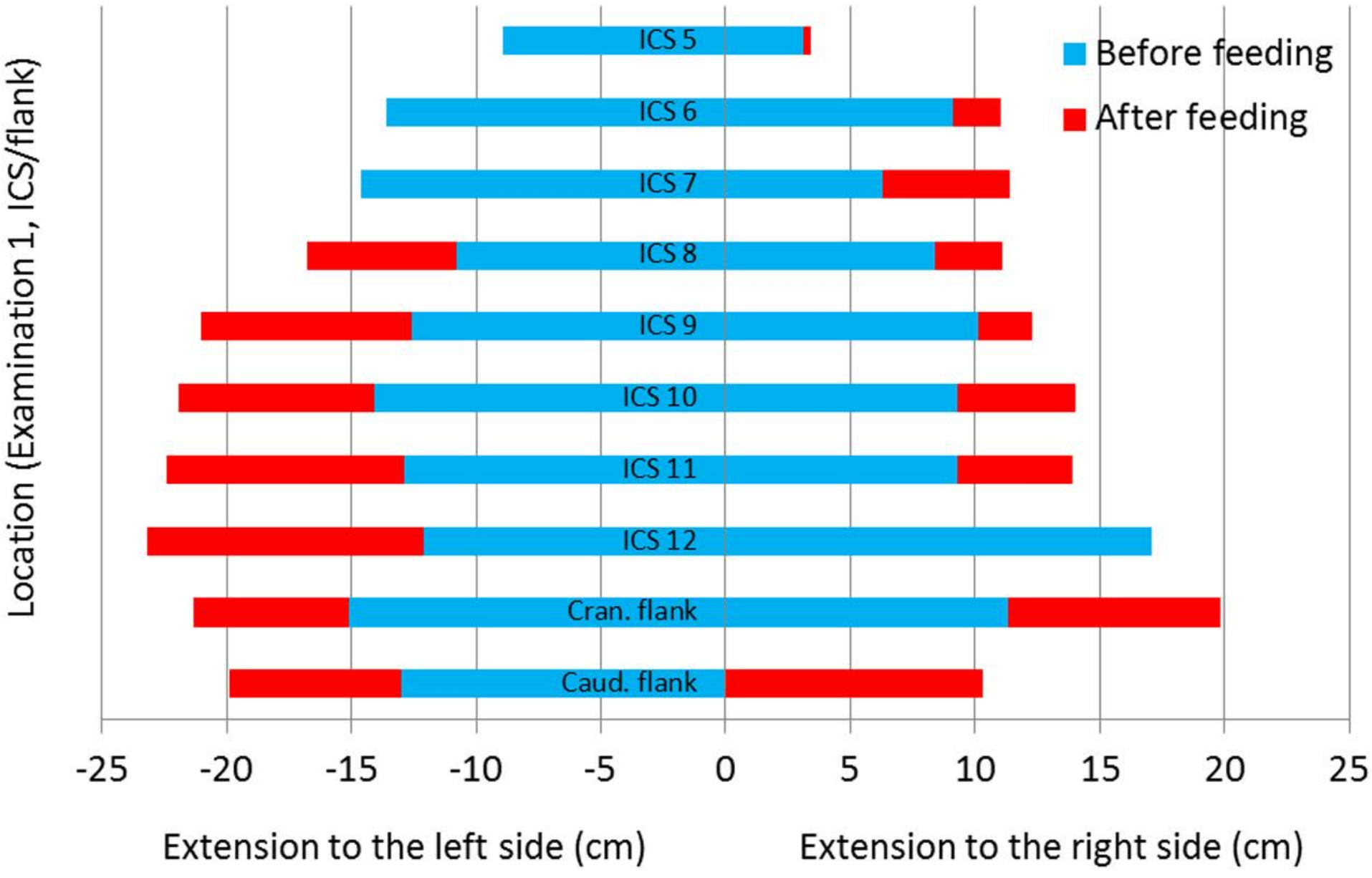

Location (intercostal spaces/flank)
7
8
9
10
11
12
Cran. flank
Caud. flank
Centimetres from the dorsal midline
0
-10
-20
-30
-40
-50
-60
Dorsal visible limit
Ventral visible limit

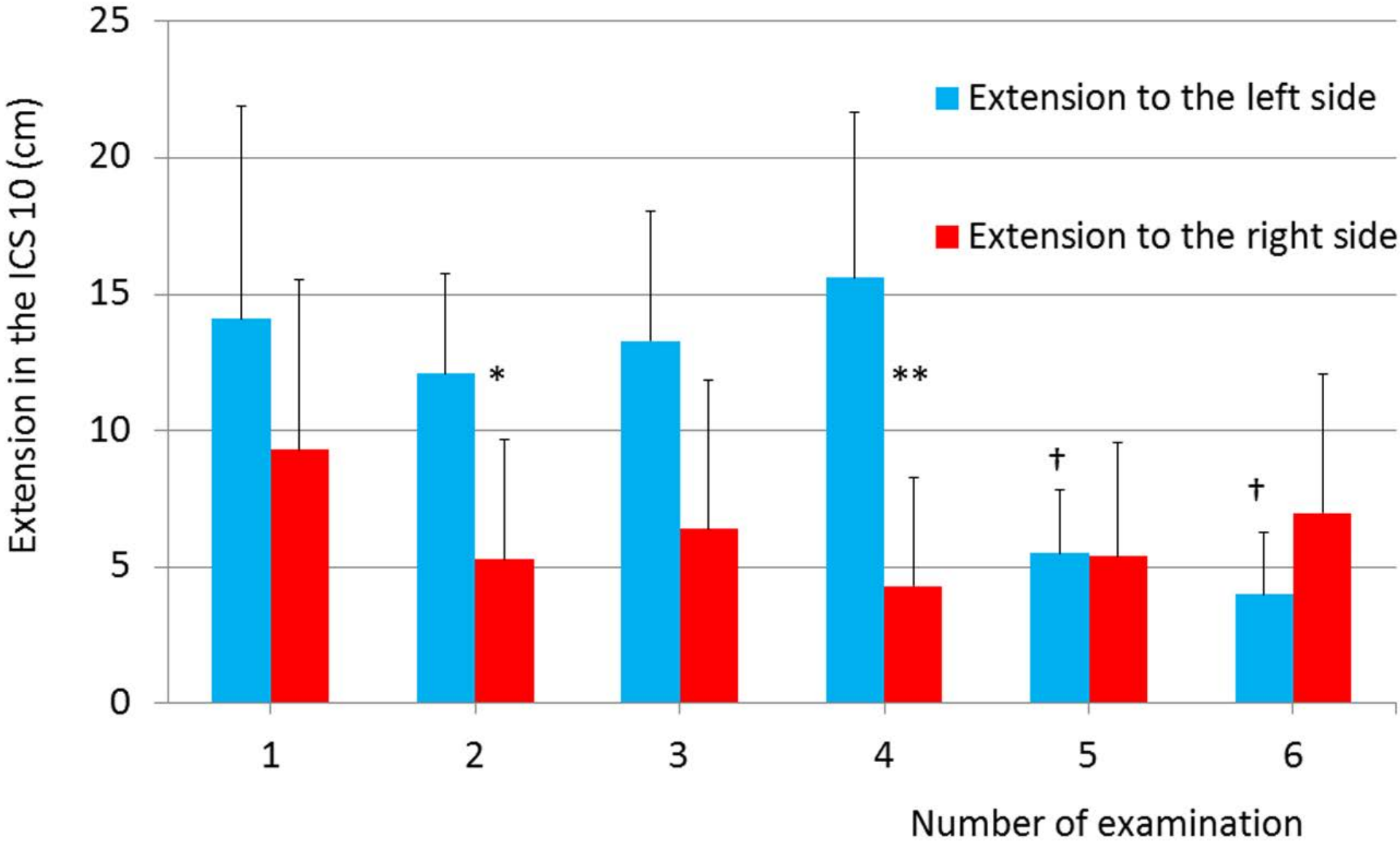

60
50
40
30
20
10
0
Size in the ICS 11 (cm)
Dorsal sac of the rumen
Ventral sac of the rumen
Rumen (dorsal and ventral sac)
1
2
3
4
5
6
Number of examination


Intercostal space
6 (n=4)
7 (n=6)
8 (n=6)
9 (n=5)
10 (n=3)
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
Centimetres from the dorsal midline
Dorsal limit of the omasum
Ventral limit of the omasum


ICS 6
ICS 7
ICS 8
ICS 9
35
30
25
20
15
10
5
0
Size of the omasum (cm)
1
2
3
4
5
6
Number of examination


Before feeding
After feeding
Length of the abomasum (cm)
40
35
30
25
20
15
10
5
0
*
*
*
a
1
2
3
4
5
6
Number of examination


Location (Examination 1, ICS/flank)
ICS 5
ICS 6
ICS 7
ICS 8
ICS 9
ICS 10
ICS 11
ICS 12
Cran. flank
Caud. flank
Before feeding
After feeding
-25
-20
-15
-10
-5
0
5
10
15
20
25
Extension to the left side (cm)
Extension to the right side (cm)


25
20
15
10
5
0
Extension in the ICS 10 (cm)
Extension to the left side
Extension to the right side
*
**
†
†
1
2
3
4
5
6
Number of examination